THE SYNTHESIS OF THE CARBONYL-^{14}C ANALOG OF ZATOSETRON MALEATE, A POTENT, LONG-ACTING, ORALLY EFFECTIVE 5-HT$_3$ RECEPTOR ANTAGONIST[1]

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SUMMARY

Endo-5-chloro-2,3-dihydro-2,2-dimethyl-N-(8-methyl-8-azabicyclo-[3.2.1.]-oct-3-yl-7-benzofurancarboxamide-[carbonyl-^{14}C] (*Z*)-2-butene-dioate (zatosetron-[^{14}C] maleate,**1**), has been prepared from 5-chloro-7-bromo-2,3-dihydro-2,2-dimethylbenzofuran (**5**) in four radiochemical steps with the reaction of **5** with K^{14}CN/ CuCN as the key step. The synthesis of **5** from 2-bromo-4-chlorophenol is also outlined.

Key words: zatosetron maleate, 5-HT$_3$ receptor antagonist, carbon 14 labeled

INTRODUCTION

Because of the wide variety of pharmacological and biochemical actions of serotonin (5-HT), it has long been suspected that there are a number of distinctly different subtypes of 5-HT receptors.[2] It has recently been determined that there are at least six 5-HT receptor subtypes.[3] A number of specific 5-HT$_3$ receptor antagonists have been identified;[4] many such agents have been implicated for use in the treatment of GI motility disorders[5], pain associated with migraines[6], anxiety[7], and emesis.[8] Lacefield *et al.*, have recently described the synthesis and structure-activity relationships of a series of 5-HT$_3$ receptor antagonists, the most active of which was *endo*-5-chloro-2,3-dihydro-2,2-dimethyl-N-(8-methyl-8-azabicyclo-[3.2.1.]-oct-3-yl-7-benzofurancarboxamide, (*Z*)-2-butenedioate (zatosetron maleate, **1**).[9] In order to facilitate pre-clinical metabolism and disposition studies of **1**, radiolabeled material was required. In this report we have described the synthesis of zatosetron-[^{14}C] maleate (**10**).

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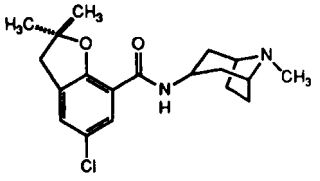
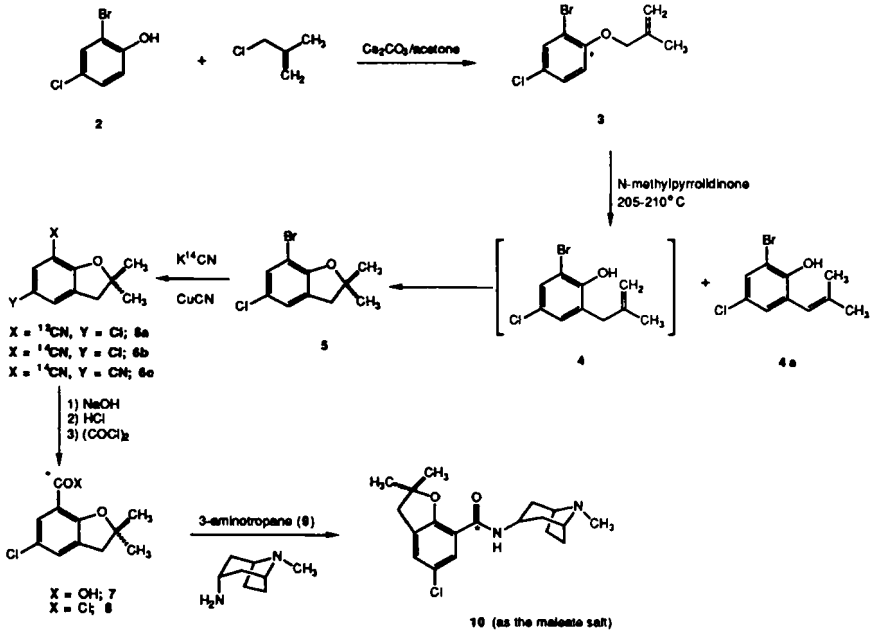


1 (as the maleate salt)

DISCUSSION

Reaction of 2-bromo-4-chlorophenol (**2**) with 2-chloro-3-methylpropene/Cs_2CO_3 in acetone provided methallyl ether **3**. Claisen rearrangement in N-methylpyrrolidinone (N-MP) and subsequent cyclization of the intermediate methallylphenol **4** without isolation resulted in the smooth conversion to 7-bromo-5-chloro-2,3-dihydro-2,2-dimethylbenzofuran (**5**). A small amount of the uncyclized conjugated olefin **4a** was isolated by flash chromatography. Using conditions described by Brown *et al*, in the synthesis of ^{14}C-labeled "spy dust"[10], reaction of **5** with a mixture of CuCN and KCN (or $K^{14}CN$) in N-MP at 210°C provided for the smooth conversion to nitrile **6a** (or the corresponding ^{14}C-analog **6b**) in 83% yield after flash chromatography, which removed a small amount of dinitrile **6c**. The nitrile **6a,b** was smoothly converted to carboxylic acid **7a,b** by treatment



in refluxing methanolic KOH. Reaction of **7b** with oxalyl chloride in toluene, followed by reaction of the resulting acid chloride **8** with *endo*-3-aminotropane[11] (**9**) and subsequent purification by flash chromatography and salt formation (maleic acid), provided **10** in 29.4% overall radiochemical yield (sp.act. = 12.3 μCi/mg or 5.71 mCi/mmol). A sample of **10** co-chromatographed with authentic **1**[9] in three TLC systems: $CHCl_3$/ CH_3OH/ NH_4OH 90:10:1, r_f = 0.35; CH_3OH/NH_4OH 98:3, r_f = 0.30; and $CHCl_3$/ acetone/NH_4OH 50:50:5, r_f = 0.55. The radiochemical purity was ≥ 98.7% in all three solvent systems.

EXPERIMENTAL

The potassium cyanide-[^{14}C] was purchased from DuPont NEN. The NMR spectra were obtained on a General Electric QE-300 nuclear magnetic resonance spectrometer at 300 MHz. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane. Direct chemical ionization mass spectra (DCI-MS) and Electron Impact mass spectra (EI-MS) were recorded on a Nermag R30-10 triple stage quadrapole mass spectrometer. High resolution FAB mass spectra were recorded on a VG Analytical VG-ZAB 3F mass spectrometer.[12] Microanalytical, IR, and UV data were provided by the Physical Chemistry Research Department of the Lilly Research Laboratories.

Flash chromatography was performed as described by Still *et al.*, using E.M. Science silica gel 60 (230-400 mesh).[13] Unless otherwise noted, the organic extracts were dried over anhydrous sodium sulfate.

Radiochemical purity was assessed by autoradiography employing E. Merck silica gel 60 F-254 TLC plates and Kodak BB-5 x-ray film. The radioactive lane was divided, suspended in methanol, and after sonication, the mixture was diluted with Amersham Corp. PCS scintillation cocktail and counted.

2-Methyl-3-(2-Bromo-4-chlorophenoxy)propene, 3:

To 25.00 g (120.5 mmol) of 2-bromo-4-chlorophenol (**2**) in dry acetone (91 mL) was added 39.66 g (121.72 mmol) of Cs_2CO_3. The suspension was vigorously stirred for 15 min and then 2-chloro-3-methylpropene (11.02 g, 127.2 mmol) was added dropwise over a 15 min period. The reaction became slightly warm during the addition. After 14 hr an additional 3mL (2.78g, 31.00 mmol) of 2-chloro-3-methylpropene was added along with 9.90 g (30.00 mmol) of Cs_2CO_3. The mixture was stirred an additional 2 hr with no further indication of product formation by TLC (chloroform/methanol 95:5). The suspension was poured into EtOAc and washed with water to remove the cesium salts. After drying the solvent was removed *in vacuo*. Initial attempts at purification by distillation were unsuccessful. The crude compound was dissolved ethyl ether (100 mL) and washed with 1N NaOH (3x25 mL) until no trace of the starting phenol was indicated by TLC. After drying, the Et_2O was removed under reduced pressure at 35°C. The yellow liquid was distilled at 132°C to 135°C at 5 mm to yield 15.00 g (48%) of **3** as a clear, colorless liquid which was a single component by TLC (r_f = 0.71): NMR ($CDCl_3$)

δ1.83 (s, 3H, -CH_3), 4.46 (s, 2H, -CH_2-), 5.01 and 5.13 (2s, 2H, =CH_2), 6.79 (d, 1H, J = 8.78 Hz, aromatic 6-H), 7.20 (dd, 1H, J = 2.52 Hz and J = 8.78 Hz, aromatic 5-H), and 7.56 ppm (d, 1H, J = 2.52 Hz, aromatic 3-H) ; EI-MS M^+ 260; UV λ_{max} (EtOH) 294 nm (ε, 2250).

Analysis calc'd for: $C_{10}H_{10}BrClO$: C, 45.92; H, 3.85. Found: C, 46.11; H, 3.85.

7-Bromo-5-chloro-2,2-dimethylbenzofuran, 5:

To 7.31 g (27.95 mmol) of ether **3** was added 10 mL of N-MP. The reaction mixture was heated under argon at 205- 210°C for 6 hr. After cooling to room temperature, the black solution was poured into 20 mL of distilled water and extracted with a mixture of 1:1 EtOAc/hexane (4x10 mL). Decolorizing charcoal was added and the solution was dried overnight. After filtration the solvent was removed under reduced pressure to yield 7.30 g of **5** as a yellow-brown liquid which was purified by flash chromatography, eluting with hexanes/EtOAc 9:1. Fractions 5-6 yielded 5.99 g (82%) of **5**; TLC (r_f = 0.45): NMR ($CDCl_3$) δ 1.50 (s, 6H, -CH_3), 3.07 (s, 2H, -CH_2-), 7.03 (bs, 1H, 4-H), and 7.26 ppm (bs, 1H, 6-H); EI-MS, M^+ 260; UV λ_{max} (EtOH) 300 nm (ε 3670).

Analysis calc'd for $C_{10}H_{10}BrClO$: C, 45.92; H, 3.85; Cl, 13.56; Br, 30.55. Found: C, 46.15; H, 3.80; Cl, 13.47; Br, 30.79.

Fraction 7 yielded a mixed fraction containing **4** and **4a**. A pure sample of **4a** was isolated by prep-TLC on silica gel (3:1 hexanes/ethyl acetate), TLC (9:1 hexanes/ethyl acetate, r_f = 0.33); NMR ($CDCl_3$) δ 1.79 and 1.90 (3H, s, CH_3), 5.50 (1H, s, OH, exchanges with D_2O), 6.19 (1H, s, =CH), 7.1 and 7.39 ppm (aromatic); DCI-MS, $(M+H)^+$ 261.

HR-FABMS calc'd for [$C_{10}H_{10}BrClO$ + H]: 260.968179. Found: 260.97200.

5-Chloro-7-cyano-2,2-dimethylbenzofuran, 6a:

To a mixture of CuCN (0.048 g, 0.54 mmol) and KCN (0.017 g, 0.26 mmol) was added 0.5 mL of N-MP. A solution of **4** (0.210 g, 0.8 mmol) in 1.5 mL of N-MP was added and the resulting mixture was heated at 210°C for 5 hr. The mixture was allowed to cool to room temperature , poured into 10% aqueous ethylenediamine, and extracted with ethyl acetate (3x10 mL). The combined extracts were dried and concentrated *in vacuo*; the residue was purified by flash chromatography. The desired product was eluted with 10 mL fractions of hexanes/ethyl acetate (3:1). Fractions 6-8 were combined and concentrated to yield **6a** as a light yellow semi-solid (0.135 g, 83%): TLC (3:1 hexanes/ethyl acetate, r_f = 0.37); UV λ_{max} (EtOH) 217 (ε 33600); FT-IR 2238 cm^{-1} (CN); DCI-MS, $(M+H)^+$ 207; NMR ($CDCl_3$) δ 1.45 (6H, s, *gem*-di-CH_3), 3.03 (2H, s, CH_2), and 7.26 ppm (2H, s, 4 and 6-H).

HR-FABMS calc'd for [$C_{11}H_{10}ClNO$ + H]: 208.05291. Found: 208.05443.

Further elution yielded 5,7-dicyano-2,2-dimethylbenzofuran (6c, 0.006 g) as a pale yellow oil; TLC (3:1 hexanes/ethyl acetate), r_f = 0.15; DCI-MS, $(M+H)^+$ 198; NMR ($CDCl_3$) δ 1.56 (6H, s, *gem*-di-CH_3), 3.10 (2H, s, CH_2), 7.54 (1H, s, 4-H), and 7.65 ppm (1H, s, 6-H), UV λ_{max} (EtOH) 220, 259, 315 (ε 30247, 12575, 4817).

HR-FABMS calc'd for [$C_{12}H_{10}N_2O$ + H]: 199.08713. Found: 199.08392.

5-Chloro-7-cyano-2,2-dimethylbenzofuran-[nitrile-^{14}C], 6b:
To a mixture of CuCN (448 mg, 4.98 mmol) and $K^{14}CN$ (144 mg, 2.22 mmol, 24 mCi/mmol, 53 mCi) in 15 mL of N-MP was added 1.88 g (7.20 mmol) of **2** in 3.0 mL of N-MP. The dark solution was refluxed under argon for 4.5 h. The solution was cooled to room temperature and 30 mL of 10% aqueous ethylenediamine was added. The solution was extracted with EtOAc (4x40 mL) and the combined organic extracts were washed with brine (1x30 mL) and were then dried. The solvent was removed under reduced pressure and the dark brown liquid was purified by flash chromatograpy eluting with 3:1 hexanes/ethyl acetate to yield **6b** (1.22 g , 82%) as an oily yellow solid. This material co-eluted with **6a** on TLC (3:1 hexanes/ethyl acetate).

5-Chloro-2,2-dimethyl-7-benzofurancarboxylic Acid-[carboxyl-^{14}C], 7:
A solution of **6b** (1.22 g, 27 mCi) in 36 mL of methanolic KOH (17.6 g of KOH dissoloved in 12 mL of water and diluted to 50 mL with methanol) was refluxed for 6 h. The solution was concentrated under reduced pressure and the white precipitate was dissolved in 95 mL of distilled water. The solution was extracted with Et_2O (3x50 mL) and the ethereal layers were discarded. The basic solution was acidified, with ice cooling, by the slow addition of 20 mL of conc. HCl. The white precipitate was then extracted with Et_2O and the combined organic extracts were dried. Filtration and removal of the solvent *in vacuo* produced **7** (1.10 g , 83%). This material was a single spot on TLC ($CHCl_3$/MeOH/HOAc, 14:3:0.5) and co-eluted with authentic material (r_f = 0.58).

5-Chloro-2,2-dimethyl-7-benzofurancarbonylchloride-[carbonyl-^{14}C] , 8:
To 1.11 g (4.9 mmol) of the acid in 45 mL of toluene was added 0.86 mL (9.8 mmol, 2 eq) of oxalyl chloride. The reaction was cooled in an ice bath and 2 drops of dimethylform-amide were added. After approximately 10 min the ice bath was removed and the reaction was stirred at room temperature for 2.5 h. The solvent was removed under reduced pressure, additional toluene was added and also removed under reduced pressure to eliminate residual aamounts of oxalyl chloride. The yellow-brown solid was dried under vacuum and utilized without further purification.

***Endo*-5-Chloro-2,3-dihydro-2,2-dimethyl-N-(8-methyl-8-azabicyclo-[3.2.1.]-oct-3-yl-7-benzofurancarboxamide-[carbonyl-^{14}C], (Z)-2-Butenedioate (LY277359-[^{14}C] Maleate, 10:**
To 1.2 g (4.9 mmol) of **8** in 35 mL of toluene was added dropwise 0.686 g (4.9 mmol) of *endo*-3-aminotropane[11] (**9**) in toluene (5 mL). The ice bath was removed and the white suspension was allowed to warm to room temperature. The solution was then refluxed

overnight. After cooling, 20 mL of 1 N HCl was added. The solution was extracted with Et_2O (3x10 mL) and the combined organic layers were discarded. The aqueous layers were then made basic by the portionwise addition of 15 mL of 2 N NaOH. The white precipitate was then extracted with $CHCl_3$ and the combined organic extracts were dried. After filtration and removal of the solvent under reduced pressure 2.21 g of crude product was obtained. The product was purified by repeated (2X) flash chromatography ($CHCl_3$/MeOH/NH_4OH, 90:10:1) to give 1.46 g (4.2 mmol, 86%) of the desired product. The amide was then dissolved in 10 mL of absolute EtOH and 0.652 g (5.6 mmol, 1.3 eq) of maleic acid was added. An additional 15 mL of EtOH was added and the mixture was refluxed until in solution. Upon cooling the solvent was carefully removed under reduced pressure. The residue was dissolved in 10 mL of refluxing EtOH and then 20 mL of EtOAc was added. After standing overnight at room temperature, the solution was cooled to 5°C and after approximately 2 h, the white crystals were collected and washed with cold EtOAc. The product was dried under vacuum overnight to yield 15,498 μCi of **10** (1.26 g, 65%, sp. act.= 12.3μCi/mg or 5.72 mCi/mmol) : DCI-MS $(M+H)^+$ 349; TLC $CHCl_3$/MeOH/ NH_4OH, 90:10:1, r_f = 0.35; MeOH/NH_4OH, 98:2, r_f = 0.30; $CHCl_3$/acetone/NH_4OH, 50:50:5, r_f = 0.55; the radiochemical purity was 98.9, 99.5, and 98.7% respectively in the three systems by autoradiography.

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